

Use of Lipid Calories During Pneumococcal Sepsis in the Rhesus Monkey

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ABSTRACT. A number of studies in patients and monkeys have suggested that intravenous infusion of lipids can be utilized as a major source of calories during sepsis and/or trauma. Others, however, have observed that lipid calories were not as effective as glucose calories in sparing body protein in severely septic and/or burned patients. In trying to resolve this controversy, a parenteral nutrition model was utilized during pneumococcal sepsis in the rhesus monkey. Earlier studies indicated that when monkeys were infused with 0.55 grams amino acid nitrogen and 13 kilocalories per kilogram per day, they lost approximately 13% of their body protein as a result of the clinical illness associated with pneumococcal sepsis. Addition of 85 kilocalories per kilogram per day of either dextrose or lipid emulsion (Intralipid) effectively prevented this infection-related loss of body protein. The present study was designed to determine whether the elevated plasma insulin concentrations associated with glucose infusions are necessary for protein sparing or would interfere with utilization of lipid calories. In this study, septic and control monkeys were infused with a hypocaloric amino acid-dextrose solution to which was added

lipid emulsion. If septic monkeys were totally maintained on a hypocaloric infusion of amino acids and 32 kilocalories per kilogram per day from dextrose, they lost $6.8 \pm 1.0\%$ of their body protein over the 6-day experimental period. Addition of lipid calories (55 kilocalories per kilogram per day) to this mixture resulted in a gain of $1.1 \pm 0.7\%$ of body protein. In both experimental groups plasma insulin was approximately 100 to 200 microunits per milliliter, which resulted in a marked inhibition of ketogenesis and lipolysis. Despite elevated plasma insulin, the septic monkey appeared able to utilize the fatty acids from the infused lipid effectively as a calorie source to spare body protein. Since lipid emulsion contains approximately 13% glycerol calories, septic monkeys were infused with a hypocaloric amino acid-dextrose solution and an amount of glycerol calories equivalent to that found in Intralipid. Under these conditions, the loss of body protein was very similar to that seen when the hypocaloric amino acid-dextrose solution alone was infused in the septic monkey. Thus, the protein-sparing effects of the lipid emulsion are not related to its glycerol content.

As shown previously in a monkey model, an intravenous infusion of amino acids (AA) and calories will prevent protein wasting associated with pneumococcal sepsis.¹ In this model, lipid calories were utilized at only slightly reduced efficiency when compared to an isocaloric infusion of dextrose. A number of investigators²⁻⁷ have observed that lipid calories in combination with AA will prevent weight loss and nitrogen (N) wasting in patients with combined sepsis and trauma. When traumatized patients were switched from a parenteral nutrition (PN) regimen that supplied the nonprotein calories as dextrose to one that contained isocaloric amounts of dextrose plus lipid, or vice versa, N balance was not significantly altered in these cross-over studies.⁶ In contrast, Long et al⁸ have observed increased protein-sparing effects of intravenous (IV) dextrose as compared to IV lipid in burned patients who were septic. IV infusion of dextrose calories was slightly better than lipid in trau-

matized patients who were highly catabolic.^{9,10} Several hypotheses have been advanced to explain the apparent controversial value of the use of IV lipid calories in critically ill patients; these include the following: (1) suggestion that glycerol content of the fat emulsion was the major caloric source utilized by burned patients¹¹; (2) that the greater protein-sparing effects of dextrose as compared to fat when infused IV in severely catabolic patients is related to the insulin response excited by the former caloric substrate^{9,12}; (3) that lipid calories are effective in treating starvation but not catabolic response to injury.¹² To test these hypotheses, a PN model in Rhesus monkey^{1,13} was utilized to evaluate the efficiency of lipid calories during pneumococcal sepsis. In this model protein-wasting is associated with the catabolic effects of the infectious disease and not simple starvation.¹ In the present study, a combined dextrose and lipid infusion was utilized to evaluate the effects on protein wasting in control and infected monkeys. This involved the infusion of a basal hypocaloric AA-dextrose solution with and without added lipid calories. To assess the contribution of the glycerol calories in the lipid emulsion, an equivalent amount of glycerol was added to the basal hypocaloric AA-dextrose solution and infused IV to control and infected monkeys.

METHODS

Thirty male Rhesus monkeys (*Macaca mulatta*), weighing 3.5 to 5.5 kg were adapted to chair-restraint and had indwelling catheters placed in the internal jugular

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TABLE I
Composition of basal hypocaloric solution for monkeys

Ingredient	Amount
	ml
8.5% AA ^b	500
50% dextrose ^c	200
Sodium chloride (25 mEq/ml) ^c	10
Potassium phosphate (4.4 mEq K, 93 mg P/ml) ^c	6
Magnesium sulfate (4.05 mEq mg/ml) ^c	4.5
Calcium gluceptate (4.5 mEq Ca/5 ml) ^c	5
Zinc chloride (1 mg Zn/ml) ^c	1
Cupric chloride (0.4 nag cu/ml) ^c	1
Chromic chloride (4 µg Cr/ml) ^c	0.5
Manganese chloride (0.1 mg mm/ml) ^c	1
Iron dextran (1 mg/ml) ^c	1
MVI ^d	10
Heparin (1000 U/ml) ^e	3
Sterile water ^f	57
Total	800

^a Solution infused at rate of 75 ml/kg/day⁻¹

^b FreAmine II, McGaw Labs, Inc, Irvine, CA.

^c Abbott Labs, North Chicago, IL.

^d The Vitarine Co, Inc, New York, NY.

^e Merrill-National Laboratory, Cincinnati, OH.

^f Multi-Vitamin infusion, USV Laboratory, Tuckahoe, NY.

^g Lyho, Med, Inc, Chicago, IL.

vein, femoral vein, and carotid artery as described previously.¹⁻¹³ On the day after surgery, a basal hypocaloric solution was infused via the jugular vein and supplied 0.55 g AA N and 13 kcal/kg/day (FreAmine II, McGaw), with 32 kcal/kg/day of nonprotein calories from dextrose plus electrolytes, trace elements, and vitamins (Table I). This basal solution was infused at the rate of 75 ml/kg/day; the infusion rates were metered by an IV infusion pump (Model No. II-D Life Care Pump, Abbott Laboratories).

At 1000 hours on the day after starting the infusion of the PN solution, the monkeys were injected via the femoral vein with 3×10^8 live or heat-killed *Streptococcus pneumoniae*, type I, strain A5. Monkeys injected with the heat-killed organisms served as controls.

The monkeys were assigned to one of three experiment groups, as follows: one was infused with the basal solution plus 50 ml/kg/day sterile water; the second consisted of the basal solution plus 50 ml/kg/day from a 10% fat emulsion (Intralipid, Cutter Laboratories); and the third consisted of the basal solution plus 50 ml/kg/day of a 3.3% glycerol solution. The infusion rate of the water, lipid emulsion, or glycerol solution was regulated by a separate IV-infusion pump (IVAC-500, IVAC Corp); they were mixed with the basic solution in a "T" connector (Abbott Laboratories), which was held in a sterile glove box. Each experimental group consisted of four controls and six infected monkeys. Mean body weights were similar for both control and infected monkeys.

Twenty-four-hr urine and feces collections were made on each monkey as described previously.^{1,13} Daily urine volumes were recorded; a specimen was analyzed for pH, glucose, protein, and ketones by dip stick (Ames Co); and samples were frozen at -20°F for analysis of total N by automated procedures.¹⁴ Fecal samples were homogenized as described previously^{1,13} and analyzed for total N content.

A zero-time fasting blood sample was obtained immediately before starting nutrient infusion and additional

samples were taken on days 1 to 3 and 6. The blood was analyzed for red and white blood cell counts. Plasma was separated from the blood and analyzed for free fatty acids,¹⁵ cholesterol,¹⁶ and triglycerides¹⁶ by automated procedures and insulin by radioimmunoassay.¹⁷ Hours of fever were calculated as a product of degrees Fahrenheit greater than 100 multiplied by duration in hours.

For sequential analysis within a group, data was analyzed by paired one-way analysis of variance. Comparison between group were analyzed by unpaired one-way analysis of variance. A p value less than 0.05 was considered significant under the null hypothesis.

RESULTS

Infusion with hypocaloric dextrose plus AA solution resulted in a marked increase in plasma insulin to approximately 200 µU/ml (Fig. 1). The addition of 55 kcal/kg/day of lipid emulsion to the hypocaloric AA-dextrose solution in control monkeys resulted in a markedly positive N balance during the 6-day experimental period (Fig. 2). In the septic monkeys, urinary N excretion was significantly increased when compared to controls. However, the monkeys tended to remain in N equilibrium or in slightly positive balance during pneumococcal sepsis. Plasma insulin concentrations were increased to approximately 200 µU/ml throughout the 6-day experimental period in both control and septic monkeys.

Since glycerol represents about 13% of the caloric value of the fat emulsion, an equivalent amount of this substrate was added to the hypocaloric AA-dextrose solution. When this mixture was infused into septic monkeys, urinary N excretion was not statistically different, by unpaired analysis of variance, from that observed when septic monkeys were infused with only the AA and 32 kcal/kg/day from dextrose (Fig. 3). Thus, the glycerol content of the fat emulsion did not appear to account for the protein-sparing action in the septic monkey.

Cumulative N balance over the 6-day period for the control and septic monkeys receiving these three different nutritional support infusions are summarized in Figure 4. These data emphasize the observations that this infection in the monkey stimulated an increased wasting of body protein. The addition of lipid calories to the hypocaloric AA-dextrose mixture reduced the wasting of body protein, but the monkeys accumulated less body protein than their noninfected controls.

By assuming that a monkey contains 15% protein which is 16% N, it was calculated that septic monkeys lost approximately 13% of their body protein if infused with 13 kcal/kg/day of AA alone (Table II). The addition of 32 kcal/kg/day dextrose significantly reduced this loss to 6.8% of their body protein. If 85 kcal/kg/day dextrose or lipid emulsion were infused together with 13 kcal/kg/day AA, protein-wasting was essentially prevented in septic monkeys.¹ Further, the addition of lipid calories to the hypocaloric dextrose-AA mixture effectively spared body protein during pneumococcal sepsis, but the addition of only the equivalent amount of glycerol calories contained in the lipid source had no significant effect on the rate of loss of body protein (Table II).

When infused with 13 kcal/kg/day AA alone, control monkeys lost approximately 2% of their body protein

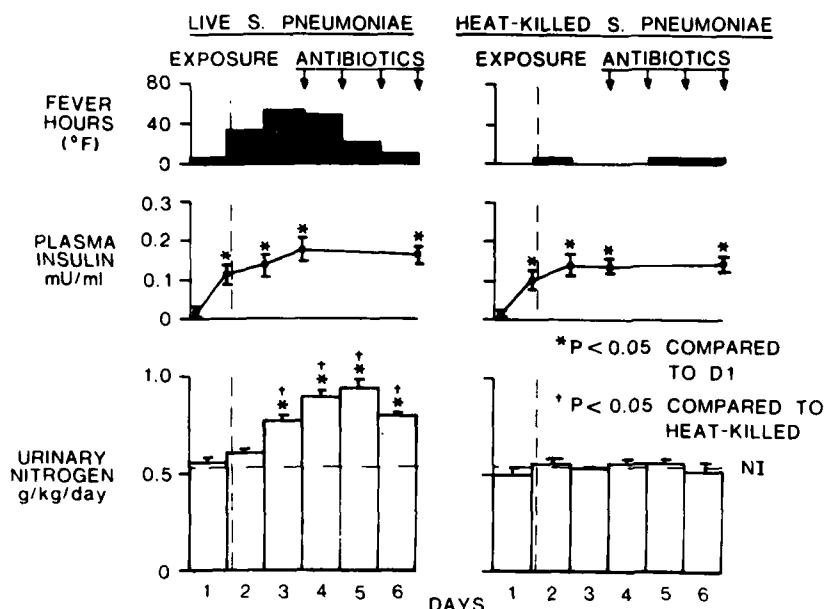


FIG. 1. Sequential changes in urinary N excretion and plasma insulin concentrations of monkeys infused with the basal AA dextrose solution. Values are mean \pm SE of six monkeys in septic group or four monkeys in control group. The horizontal dashed line is the mean N intake (NI).

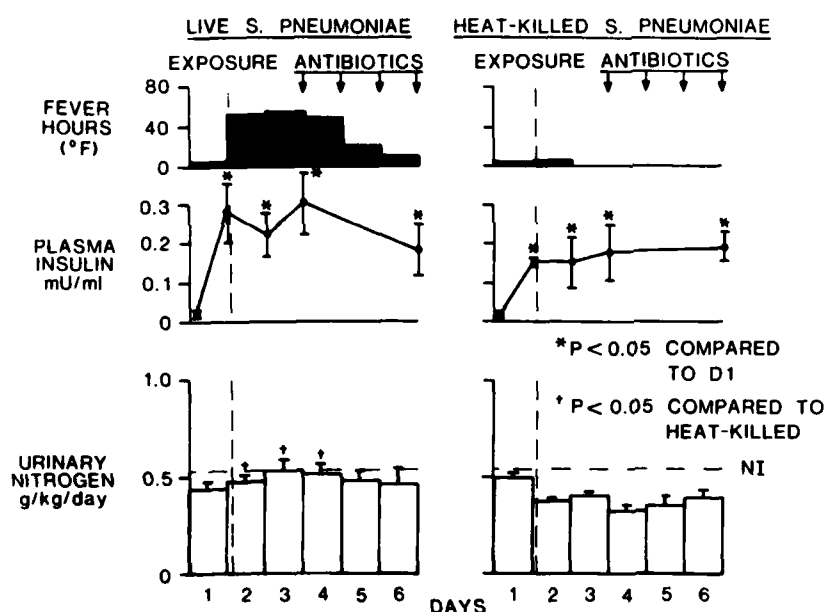


FIG. 2. Sequential changes in urinary N excretion and plasma insulin concentrations of monkeys infused with basal AA-dextrose solution plus lipid emulsion. Values are mean \pm SE of six monkeys in septic group or four monkeys in control group. The horizontal dashed line is the mean N intake (NI).

over the 6-day experimental period (Table II). The addition of 32 kcal/kg dextrose to the AA mixture essentially maintained control monkeys in N equilibrium (Table II). The addition of more dextrose or lipid calories did not significantly improve N retention in the control monkeys. Thus, a chaired nonseptic monkey was maintained in N equilibrium with approximately 45 kcal/kg/day (32 from dextrose, 13 from AA), while the septic monkey, during febrile illness, was in slight negative balance when infused with approximately 100 kcal/kg/day.

The infusion of 32 kcal/kg/day dextrose resulted in only a slight increase in plasma glucose (Fig. 5). Plasma urea N was not significantly altered by the AA infusion

with various caloric substrates (Fig. 5). The infusion of the hypocaloric AA-dextrose solution resulted in a significant decrease in plasma triglycerides, cholesterol, β -hydroxybutyrate, and free fatty acids (Fig. 5). The addition of lipid calories to this solution resulted in a significant increase in plasma triglycerides, cholesterol, and free fatty acids, but not in the β -hydroxybutyrate. Similar effects of dextrose or lipid calories were observed in both septic and control monkeys.

DISCUSSION

In the monkey model utilized in these studies, pneumococcal sepsis resulted in a marked catabolic response

FIG. 3. Sequential changes in urinary N excretion and plasma insulin concentrations of monkeys infused with basal AA-dextrose solution plus glycerol equivalent to that found in lipid emulsion. Values are mean \pm SE of six monkeys in septic group or four monkeys in control group. The horizontal dashed line is the mean N intake (NI).

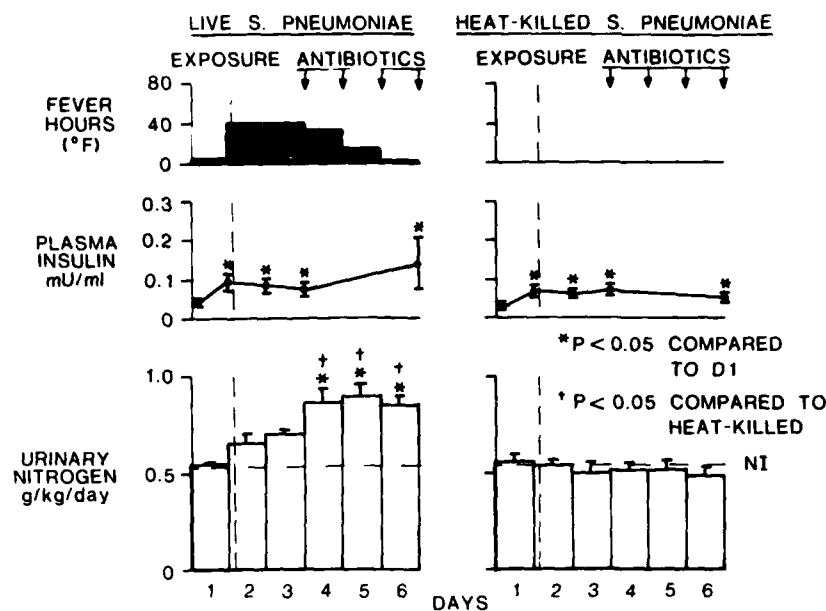
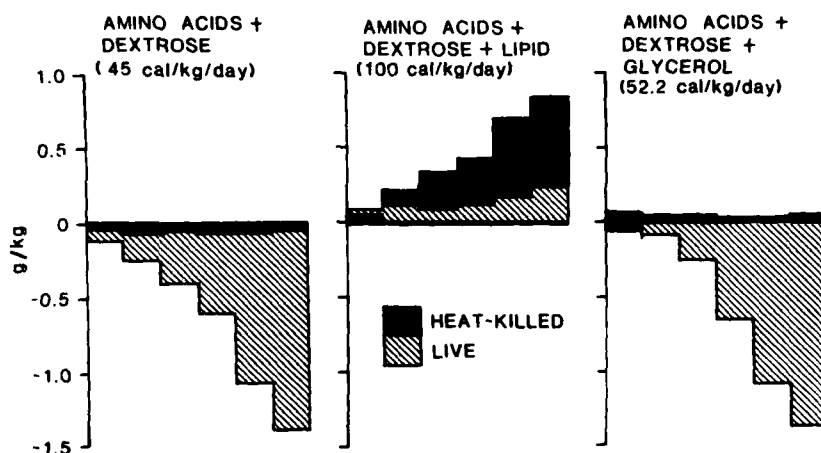


FIG. 4. Cumulative N balance over the 6-day period for heat-killed controls (■) and septic (□) monkeys receiving either basal AA-dextrose solution with or without the addition of lipid emulsion or glycerol calories.



when compared to findings in noninfected controls. The protein wasting associated with this infectious disease could be prevented by adequate infusion of AA and calories from either dextrose or lipid emulsion. These results are in agreement with others¹⁻⁷ that lipid calories can be utilized by the injured host to prevent wasting of body protein. In contrast, Long et al⁸ observed that critically ill or severely burned patients were unable to utilize lipids for protein sparing. Similar reduced efficiency of utilization of lipid calories by critically ill patients has been suggested by others.^{9,10} McDougal et al¹¹ have postulated that the glycerol content of the fat emulsion was the only major calorie source utilized by burned patients. When septic monkeys were infused with a hypocaloric solution of dextrose and AA plus the amount of glycerol found in the lipid emulsion, protein wasting in septic monkeys was similar to that observed with AA-dextrose solution alone. Thus, in this model the protein-sparing effects of lipid emulsion were not related to its glycerol content.

It has also been argued that dextrose calories are more efficiently utilized than lipid calories because of the insulin response elucidated by infusing high concentrations of glucose.^{9,11} When septic or control monkeys were infused with the 32 kcal/kg/day dextrose and AA with or without lipid emulsion, plasma insulin concentrations were increased 10 to 20-fold over those observed in fasted monkeys. There appeared to be no significant difference in protein-sparing effects of an isocaloric mixture of AA plus dextrose and lipid emulsion or AA plus dextrose.

In an earlier study, infusion of AA plus lipid emulsion alone¹ was slightly less than an isocaloric amount of dextrose plus AA in sparing body protein in septic monkey. While the difference between the two dietary regimens was not statistically significant, it did represent 2.9% body protein, which could be important from a physiologic point of view. Since the reduced protein-sparing effects of the lipid emulsion infusion was associated with a lower plasma insulin concentration, it could be argued that elevated plasma insulin concentrations

TABLE II
Per cent change in body protein during 6 day experiment

Diet	%
<i>Infected</i>	
*1 Amino acids (0.55 g N and 13 cal/kg/day)	-12.8 ± 2.7 ^{b,c,d,e,f,g,h,i,j,k,l}
*2 Amino acids + dextrose (85 cal/kg/day) ¹	1.1 ± 0.7 ^{a,c,f}
*3 Amino acids + dextrose (32 cal/kg/day)	-6.8 ± 1.0 ^{a,b,d,e,g,h,i,j,k,l}
*4 Amino acids + lipid (85 cal/kg/day)	-1.8 ± 0.6 ^{a,c,f,h,i}
5 Amino acids + dextrose (32 cal/kg/day) + lipid (55 cal/kg/day)	1.1 ± 0.7 ^{a,c}
6 Amino acids + dextrose (32 cal/kg/day) + glycerol (7.2 cal/kg/day)	-6.5 ± 0.5 ^{a,b,d,e,g,h,i,j,k,l}
<i>Heat-killed control</i>	
*7 Amino acids	-2.3 ± 0.7 ^{a,c,f,h,k}
*8 Amino acids + dextrose (85 cal/kg/day)	2.5 ± 0.6 ^{a,c,f,g}
9 Amino acids + dextrose (32 cal/kg/day)	-0.2 ± 1.4 ^{a,c,f}
*10 Amino acids + lipid (85 cal/kg/day)	1.1 ± 0.4 ^{a,c,f}
11 Amino acids + lipid (55 cal/kg/day) + dextrose (32 cal/kg/day)	4.1 ± 0.7 ^{a,c,d,f,g}
12 Amino acid + dextrose (32 cal/kg/day) + glycerol (9.4 cal/kg/day)	0.2 ± 0.3 ^{a,c,f,g}

^a p < 0.05 compared to group 1.

^b p < 0.05 compared to group 2.

^c p < 0.05 compared to group 3.

^d p < 0.05 compared to group 4.

^e p < 0.05 compared to group 5.

^f p < 0.05 compared to group 6.

^g p < 0.05 compared to group 7.

^h p < 0.05 compared to group 8.

ⁱ p < 0.05 compared to group 9.

^j p < 0.05 compared to group 10.

^k p < 0.05 compared to group 11.

^l p < 0.05 compared to group 12.

¹ The data for groups 1, 2, 4, 7, 8, and 10 from Wannemacher et al.¹

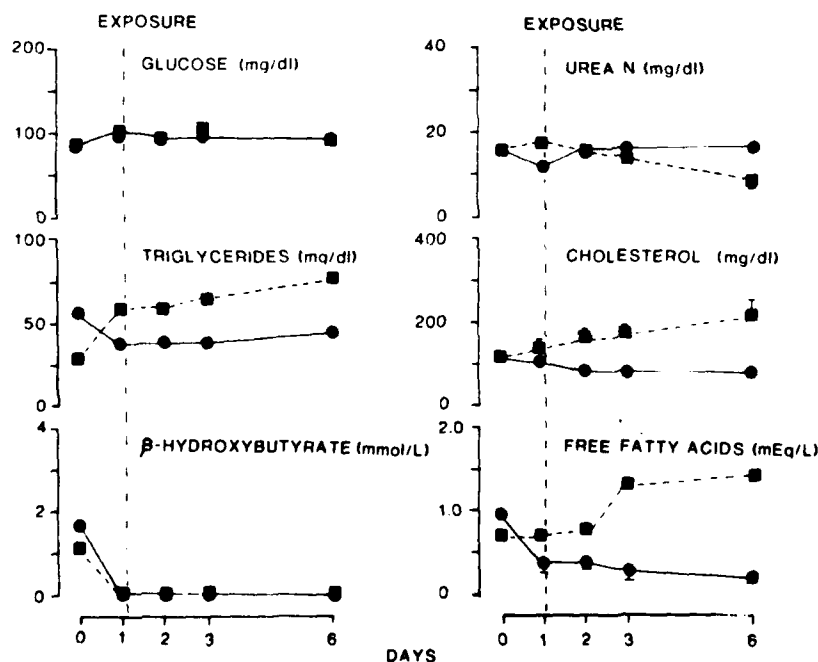


FIG. 5. Sequential changes in plasma glucose, urea N, triglycerides, cholesterol, β -hydroxybutyrate, or free fatty acids of septic monkeys infused with either basal AA solution (●) or basal solution plus lipid emulsion (■). Zero time values are from monkeys fasted for 24 hr. Each value is the mean for six monkeys and the vertical lines are SE of means that are significant (p < 0.05) different for fasting values.

tend to reduce the wasting of body protein. Thus, during pneumococcal sepsis in monkeys, high plasma insulin concentration did not prevent the utilization of lipid calories and may have been beneficial in preventing wasting of body protein.

Plasma cholesterol was progressively increased in the monkeys infused with the lipid, dextrose, and AA. Similar increases in plasma cholesterol have been observed in patients and monkeys infused with AA plus lipid or AA plus lipid and glucose solution.^{1,6} The higher plasma

cholesterol value and elevated free fatty acids support the concept that the infused long-chain triglycerides are being utilized in metabolic processes by both septic and control monkeys. Further, this utilization of infused lipid appears to take place in the presence or absence of high plasma insulin concentrations. The high plasma insulin concentrations, however, blocked the ketone body production from the long-chain fatty acids. These observations are in agreement with the postulate¹⁸ that insulin blocks hepatic production of ketones.

REFERENCES

1. Wannemacher RW Jr, Kaminaki MV Jr, Neufeld HA, et al: Protein-sparing therapy during pneumococcal sepsis in rhesus monkeys. JPEN 2:507-518, 1978
2. Yeo MT, Gazzaniga AB, Bartlett RH, et al: Total intravenous nutrition. Experience with fat emulsions and hypertonic glucose. Arch Surg 106:792-796, 1973
3. Zohrab WJ, McHattie JD, Jeejeebhoy KN: Total parenteral alimentation with lipid. Gastroenterology 64:583-592, 1973
4. Deitel M, Kaminsky V: Total nutrition by peripheral vein—the lipid system. Can Med Assoc J 111:152-154, 1974
5. Hansen LM, Hardie BS, Hidalgo J: Fat emulsion for intravenous administration: clinical experience with Intrapid 10%. Ann Surg 184:80-88, 1976
6. Jeejeebhoy KN, Anderson, GH, Nakhooa AF, et al: Metabolic studies in total parenteral nutrition with lipid in man. Comparison with glucose. J Clin Invest 57:125-136, 1976
7. Silberman H, Freehauf M, Fong G, et al: Parenteral nutrition with lipids. JAMA 238:1380-1382, 1977
8. Long JM III, Wilmore DW, Mason AD Jr, et al: Effect of carbohydrate and fat intake on nitrogen excretion during total intravenous feeding. Ann Surg 185:417-422, 1977
9. Woolfson AMJ, Heatley RV, Allison SP: Insulin to inhibit protein catabolism after injury. N Engl J Med 300:14-17, 1979
10. Dresler C, Law D, Van Way C: A comparison of glucose and fat emulsion as a caloric source in total parenteral nutrition. Clin Res 27:31A, 1979
11. McDougal WS, Wilmore DW, Pruitt BA Jr: Effect of intravenous near osmotic nutrient infusions on nitrogen balance in critically ill patients. Surg Gynecol Obstet 145:408-414, 1977
12. Allison SP: Effect of insulin on metabolic response to injury. JPEN 4:175-179, 1980
13. Wannemacher RW Jr, Hadick CL Jr, Beisel WR: Nutrition and infection interrelationships in monkeys. IN Primates in Nutritional Research, KC Hayes (ed). Academic Press, New York, 1979, pp 315-340
14. Ferrari A: Nitrogen determination by continuous digestion and analysis system. Ann NY Acad Sci 87:792-800, 1960
15. Dalton C, Kowalski C: Automated colorimetric determination of free fatty acids in biologic fluids. Clin Chem 13:744-751, 1967
16. Rush RL, Leon L, Turrell J: Automated simultaneous cholesterol and triglyceride determination on the AutoAnalyzer II instrument. IN Advances in Automated Analysis: Technicon International Congress, chap 1. Thurman Associates, Miami, 1970, pp 503-507
17. Juan C, Avruskin TW: A combined immunoassay of human growth hormone and insulin: cumulative assessment of assay performance. J Clin Endocrin Metab 33:150-152, 1971
18. Neufeld HA, Pace JG, Kaminski MV, et al: A probable endocrine basis for the depression of ketone bodies during infectious or inflammatory state in rats. Endocrinology 107:596-601, 1980

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